



**UNIVERSITI PUTRA MALAYSIA**

***ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES IN *Homalomena joseffi* BOYCE AND WONG EXTRACT AND ITS PHYTOCHEMICALS***

**KIMBERLEY RINAI RADU**

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By

**KIMBERLEY RINAI RADU**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Master of Science**

**October 2020**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**October 2020**

**Chairman : Yaya Rukayadi, PhD**  
**Institute : Bioscience**

Poisonings donned from consuming daily dietary products ranging from carbohydrates to fibrous foods is a common problem faced by every country collectively. Mainly due to poor food handling, pathogenic strains are passed on from person to person causing growing concerns in the battle against antibiotic resistant bacteria. For many years the *Homalomena* species is widely used in treating various ailments in traditional medicine, ranging from headaches to bacterial infections in Asian countries such as India and China. Recently, more *Homalomena* species have been found within the tropical forests of Borneo, including *Homalomena joseffi* P.C. Boyce and S.Y. Wong. It serves as an alternative natural antimicrobial to the local people. The aims of this study were to determine antibacterial and antioxidant activities of *H. joseffi* crude extracts and its fractions and to analyse the phytochemical constituents in *H. joseffi* extracts. The dried rhizome of *H. joseffi* was extracted using maceration methods with methanol as a solvent. The crude extract then was fractionated using liquid-liquid fractions with solvents of hexane, chloroform, ethyl acetate and aqueous methanol. The antibacterial activity of the crude extract and its fractions were tested against selected foodborne pathogens in term of disc diffusion assay (DDA), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill curve assay using standard methods of Clinical and Laboratory Standard Institute (CLSI). The total phenolic compounds (TPC) in selected extract and fractions were analysed using the Folin-Ciocalteu method. The antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The active antibacterial compounds in crude and fractions were identified by Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS). Diameter of inhibition zones of crude extracts against selected foodborne pathogens were ranged from  $9.33 \pm 0.57$  to  $11.00 \pm 0.57$  mm. MICs of the crude extract were in the range of 0.31 to 5.00 mg/mL. The crude extracts can kill the tested bacterial with MBC values in the range from 0.63 to 5.00 mg/mL. Time-kill assay curve analyses results showed that the crude extracts were able to completely kill the bacterial growth at  $4 \times \text{MIC}$  at 4 hours. The total phenolic content in the crude extract was 432.138 GAE/g. While DPPH results displayed  $\text{IC}_{50}$  of the crude extract was 11.809  $\mu\text{g/mL}$  of dried weight material. The inhibition zone of fractions from methanolic crude extract was ranged from  $7.00 \pm 0.00$  mm to  $12.50 \pm 1.00$  mm. The

MICs of fractions were range from 0.156 mg/mL to 1.250 mg/mL. Moreover, the MBCs values of fractions were ranged from 0.625 mg/mL to 5.00 mg/mL. GC-MS analyses of the crude extract resulted nine major compounds which contribute to antibacterial activity, namely hotrienol, mullilam diol, palmitic acid, viridiflorol, neryl acetate, ethyl palmitate, globulol, linoleic acid and ethyl linoleate. Whereas its fraction consists of seven major compounds such as linalool, 1-terpinen-4-ol, cryptone,  $\alpha$ -terpineol, elemol, spathulenol and methyl palmitate. Furthermore, LC-MS has identified 11 non-volatile compounds, namely corymboside, ethylmorphine, abscisic acid, sinomenine, leucylproline, rhoifolin, glutethimide, indole-2-acrylic acid, umbelliferone, quercetin and scopletin. In conclusion, *H. joseffi* extract and its compounds display potential antibacterial activity against foodborne pathogens and antioxidant activity. Therefore, it could be developed and implemented as natural food preservatives.

**Keywords:** antibacterial activity, antioxidant activity, *Homalomena joseffi*, GC-MS, LC-MS

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**AKTIVITI ANTIBAKTERIA DAN ANTIOKSIDAN DARIPADA EKSTRAK  
*Homalomena joseffi* BOYCE DAN WONG DAN ANALISIS FITOKIMIA**

Oleh

**KIMBERLEY RINAI RADU**

**Oktober 2020**

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Keracunan yang disebabkan oleh pengambilan produk makanan harian mulai dari karbohidrat hingga makanan berserat adalah masalah biasa yang dihadapi oleh setiap negara secara kolektif. Terutama kerana pengendalian makanan yang buruk, strain patogen ditularkan dari orang ke orang menyebabkan kebimbangan yang semakin meningkat dalam pertempuran melawan bakteria tahan antibiotik. Selama bertahun-tahun, spesies *Homalomena* banyak digunakan dalam merawat pelbagai penyakit dalam perubatan tradisional, mulai dari sakit kepala hingga jangkitan bakteria di negara-negara Asia seperti India dan China. Baru-baru ini, lebih banyak spesies *Homalomena* telah dijumpai di hutan tropika di Borneo, termasuk *Homalomena joseffi* P.C. Boyce dan S.Y. Wong. Ia berfungsi sebagai alternatif antimikroba semula jadi bagi penduduk setempat. Tujuan kajian ini adalah untuk menentukan aktiviti antibakteria dan antioksidan ekstrak kasar *H. joseffi* dan pecahannya dan menganalisis unsur fitokimia dalam ekstrak *H. joseffi*. Rimpang kering *H. joseffi* diekstrak menggunakan kaedah maserasi dengan metanol sebagai slovent. Ekstrak kasar kemudian difraksinasi menggunakan pecahan cecair-cecair dengan pelarut heksana, kloroform, etil asetat dan metanol berair. Kegiatan antibakteria ekstrak mentah dan pecahannya diuji terhadap patogen bawaan makanan terpilih dari segi ujian penyebaran cakera (DDA), kepekatan perencatan minimum (MIC), kepekatan minimum bakterisida (MBC), dan ujian keluk pembunuhan masa menggunakan kaedah standard Institut Standard Clinical dan Makmal (CLSI). Keseluruhan sebatian fenolik (TPC) dalam ekstrak dan pecahan terpilih dianalisis menggunakan kaedah Folin-Ciocalteu. Kegiatan antioksidan ditentukan menggunakan ujian pemuliharaan radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH). Sebatian antibakteria aktif dalam minyak mentah dan pecahan dikenal pasti oleh Gas Chromatography-Mass Spectrometry (GC-MS) dan Liquid Chromatography-Mass Spectrometry (LC-MS). Diameter zona perencatan ekstrak kasar terhadap patogen bawaan makanan terpilih berada dalam jarak antara  $9,33 \pm 0,57$  hingga  $11,00 \pm 0,57$  mm. MIC ekstrak kasar berada dalam lingkungan 0.31 hingga 5.00 mg/mL. Ekstrak kasar dapat membunuh bakteria yang diuji dengan nilai MBC dalam lingkungan 0,63 hingga 5,00 mg/mL. Hasil analisis keluk ujian membunuh masa menunjukkan bahawa ekstrak kasar dapat membunuh pertumbuhan bakteria sepenuhnya pada  $4 \times$  MIC pada 4 jam. Jumlah kandungan fenolik dalam ekstrak kasar adalah 432.138 mg GAE/g. Sementara hasil DPPH menunjukkan

IC<sub>50</sub> ekstrak kasar adalah 11.809 µg/mL bahan berat kering. Zon penghambatan pecahan dari ekstrak kasar metanol adalah antara 7.00 ± 0.00 mm hingga 12.50 ± 1.00 mm. MIC pecahan antara 0.156 mg/mL hingga 1.250 mg/mL. Selain itu, nilai pecahan MBC berkisar antara 0.625 mg/mL hingga 5.00 mg/mL. Analisis GC-MS ekstrak kasar menghasilkan sembilan sebatian utama yang menyumbang kepada aktiviti antibakteria, iaitu hotrienol, mullilam diol, asid palmitik, viridiflorol, neryl asetat, etil palmitat, globulol, asid linoleat dan etil linoleat. Manakala pecahannya terdiri daripada tujuh sebatian utama seperti linalool, 1-terpinen-4-ol, cryptone, α-terpineol, elemol, spathulenol dan metil palmitat. Selanjutnya, LC-MS telah mengenal pasti 11 sebatian tidak mudah menguap, iaitu corymboside, ethylmorphine, acid abscisic, sinomenine, leucylproline, rhoifolin, glutethimide, indole-2-acrylic acid, umbelliferone, quercetin dan scopletin. Sebagai kesimpulan, ekstrak *H. joseffi* dan sebatianannya menunjukkan potensi aktiviti antibakteria terhadap patogen bawaan makanan dan aktiviti antioksidan. Oleh itu, ia dapat dikembangkan dan dilaksanakan sebagai pengawet makanan semula jadi.

**Kata kunci:** aktiviti antibakteria, aktiviti antioksidan, *Homalomena joseffi*, GC-MS, LC-MS

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
AMR	Antimicrobial Resistance
CHX	Chlorhexidine
CLSI	Clinical and Laboratory Standards Institute
CFU	Colony forming unit
CC	Column Chromatography
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EoNPs	Essential Oil Nanoparticles
GC-MS	Gas Chromatography – Mass Spectrometry
GRAS	Generally Regarded as Safe
HPLC	High Performance Liquid Chromatography
LC-MS	Liquid Chromatography – Mass Spectrometry
<i>m/z</i>	Mass/charge ratio
MHA	Mueller Hinton agar
MHB	Mueller Hinton broth
PTLC	Preparative thin layer chromatography
ppm	part per million
PBS	Phosphate Buffer Saline
K <sup>+</sup>	Potassium cation
<sup>1</sup> H NMR	Proton Nuclear Magnetic Resonance
rpm	Revolutions per minute
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
TLC	Thin layer chromatography
UV	Ultraviolet
VLC	Vacuum liquid chromatography
DPPH	1,1-diphenyl-2-picryl-hydrazyl
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide

# CHAPTER 1

## INTRODUCTION

### 1.1 Background

In the hopes of a more holistic approach, researchers are constantly searching for various natural products derived from local plants in order to obtain its medicinal properties which would be able to cure those suffering from ailments such as chronic stomach illness, fever, inflammatory problems and more. The present uses of tropical plants are common among the numerous indigenous groups in Borneo. Hence, methods in controlling microbial proliferation by comparing the utilization of synthetic and natural antimicrobial agents are imperative.

The emergence and proliferation of bacteria which is resistant to multiple microbial drugs is a critical threat to humans, animals as well as the environment. There has been strong international consensus for actions against antimicrobial resistance (AMR), established by the United Nations General Assembly (World Health Organization, 2018). This is due to the AMR found in animal-derived bacteria that have typically surfaced from food-producing animals rather than clinical isolates from deceased animals (Badger *et al.*, 2018). According to the World Health Organization (2018), antimicrobial resistance naturally occurs at an accelerated rate because of misuse or overuse in humans and animals when prescribed without professional oversight. These include the intake of antibiotics when viral infections occur such as cold and flu. Nevertheless, chemical synthetic preservatives in food have many carcinogenic and teratogenic attributes, including residual toxicity (Shi *et al.*, 2018).

Food poisoning is one of the leading causes of illness that leads to fatalities, as they are easily contracted from person to person when people lack the knowledge of personal hygiene. Bacteria such as the *Salmonella* spp. and *Escherichia coli* O157:H7 pose the greatest threat to human health as they are considered high-risk via food for the transmission of antimicrobial resistance genes to human bacteria (Turnidge *et al.*, 2007). In addition to that, *Listeria monocytogenes* (*L. monocytogenes*) is collectively recognised as a foodborne pathogen of concern worldwide in the food industries. It may be relatively rare but reports have shown serious diseases with high fatality rates compared to other foodborne microbial pathogens (Ponniah *et al.*, 2010). In Malaysia, *L. monocytogenes* are viable in seafood, poultry and vegetables, therefore the possibilities of it remaining undetected due to the non-specific clinical manifestations and difficulty in identifying it increases at an exponential rate (Ponniah *et al.*, 2010).

An alternative cause to food poisoning is spoilage. Products contaminated with pathogens not only lead to the reduction of its quality but also generates illness and diseases (Jacob *et al.*, 2010). Several food preservation methods, namely heating, refrigeration and chemical antimicrobial spiking can reduce the risk of food poisoning outbreaks. However, these methods are also attributed to the loss of nutrients (Valero & Frances, 2006). Throughout the process of preservation, it is equally vital to ensure satisfactory consumer demand of fresh, safe food, those are free from chemical-additives. Hence, there is escalating pressure in developing natural food preservation and processing through enzymes, microbial preservatives isolated from starter cultures and plant resources (Rukayadi *et al.*, 2009).

In correspondence to the above matter, essential oils have always been front lining the industries of natural food preservatives and alternative remedies for treatments of food ailments (Schuenzel & Harrison, 2002). Numerous studies have demonstrated antimicrobial properties of plants such as the *Eucalyptus* species against a wide range of microorganisms (Elaissi *et al.*, 2011). In addition to that, the *Homalomena* species is a popular medicinal plant in tropical countries especially China. In Malaysia, the decoction of the roots and leaves has been extensively utilized as traditional medicine because of its therapeutic properties and aromatic aroma (Wong *et al.*, 2006). Bioactive compounds from *Homalomena joseffi* has not been studied but has promising effects equivalent to its counterparts such as *Homalomena aromatica*, *Homalomena occulta* and more. These bioactive compounds are responsible for anti-inflammatory, antioxidant and antimicrobial effects (Zhang *et al.*, 2018).

Plants consist of essential oils that are either utilized as medicine or as aroma therapy. Antimicrobial properties from essential oils have been recognized for centuries and have been well documented (Shantikumar *et al.*, 2006). Besides that, interests in these oils have increased due to its utilization in the eradication of methicillin-resistant *Staphylococcus aureus* (MRSA) (Carson *et al.*, 1995). An example associated with the use of essential oil from rhizomes of *Homalomena aromatica* is its efficacy against skin diseases. The yellow extract has also been recorded in the use of perfume (Nwosu & Okafor, 1995). These organic products, in its pure form or as standardized extracts allow opportunities for new drug discoveries due to the limited availability of chemical diversity (Cosa *et al.*, 2006). Many antimicrobial compounds derived from plant extracts have a broad spectrum against foodborne bacteria (Aumeeruddy-Elalfi *et al.*, 2016).

## 1.2 Problem Statements

Natural products derived from plant sources are widely explored and have been favoured by consumers as they not only exhibit a large range of antibacterial properties against microorganisms, including pathogenic microbes, but they have also been sourced as flavouring and odourising agents in food products which have piqued the interest of consumers. They have long been medicinally applied to combat the wide ranges of resistant bacterial strains that are evolving and adapting from our efforts in modern medicine. Our verdures are in abundance on our planet and it is crucial to habitually find

interest in extracting and applying these resources in the medicinal industry. These biological products have always been medically sought after by our ancestors and continue to be the main source of curing multiple ailments. In addition to that, the use of these products has expanded to the purging of microbial activities responsible for food spoilage and toxic food poisoning towards humans. The treatment of food products beginning from its source is vital for our survival and by extending the expiry dates of food products, food sources will never be scarce and eliminates the problem of storing comestibles in factories and shipping internationally. Discovering natural additives with antimicrobial activities to prevent growth during transportation is imperative as natural products have been investigated to have fewer side effects when consumed compared to modern synthetic medicine even though the latter exhibits better properties. This has inspired the present study to determine the limitations in different types of solvent used in extractions and fractions against multiple types of bacteria.

### 1.3 Objectives

The objectives of this study are:

1. To determine antibacterial activity of *Homalomena joseffi* rhizome extracts against selected foodborne pathogens.
2. To determine the total phenolic compound (TPC) and antioxidant activities of selected extracts of *Homalomena joseffi*.
3. To identify bioactive compounds in *Homalomena joseffi* rhizome extract and fractions responsible for antibacterial and antioxidant activities through several chromatography methods.

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The student, Kimberley Rinai Radu, was born on the 23<sup>rd</sup> of April 1996 in Selangor, Malaysia. She underwent primary and secondary education in public schools within the city of Kuala Lumpur from 2003 to 2017. In 2015, she enrolled into Universiti Malaysia Sarawak (UNIMAS) for her bachelor's degree in science. She applied for internship in 2017 at the Sarawak Tropic Peat Research Institute (STROPI) in Kota Samarahan, Sarawak, as a junior lab assistant to gain more knowledge in the field of research and development. Graduating with her class in 2018, she obtained a Degree in Resource Chemistry. After graduation, she was granted admission in the Institute of Bioscience University Putra Malaysia (UPM) to pursue her Master's program under the supervision of the esteemed Associate Professor Dr Yaya Rukayadi in the field of Phytochemistry.



**Proceedings:**

1. Kimberley Rinai Radu, Intan Safinar Ismail, Son Radu, Yaya Rukayadi. Anti-microbial and Anti-oxidant of *Homalomena sagittifolia* J. extract. International Food Research Journal Conference 2019 (poster presentation). Putrajaya, Malaysia.
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## LIST OF PUBLICATION

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